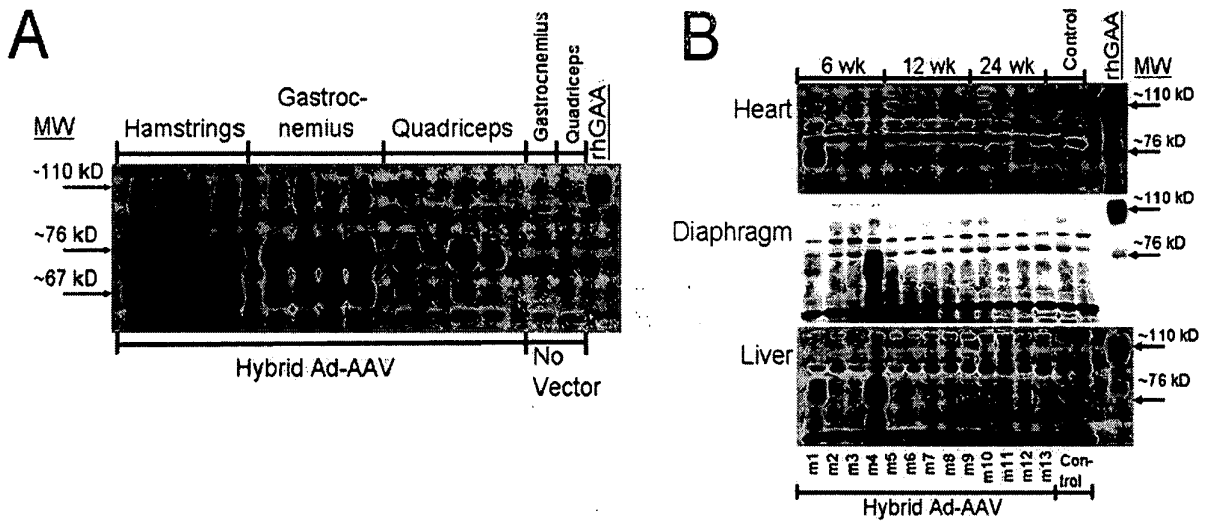

A
MW
Hamstrings
Gastroc-
nemius
Quadriceps
Gastrocnemius
Quadriceps
rhGAA
~110 kD
~76 kD
~67 kD
Hybrid Ad-AAV
No
Vector
B
6 wk
12 wk
24 wk
Control
rhGAA
MW
Heart
~110 kD
~76 kD
~110 kD
Diaphragm
~76 kD
Liver
~110 kD
~76 kD
m1
m2
m3
m4
m5
m6
m7
m8
m9
m10
m11
m12
m13
Con-
trol
Hybrid Ad-AAV

FIG. 1

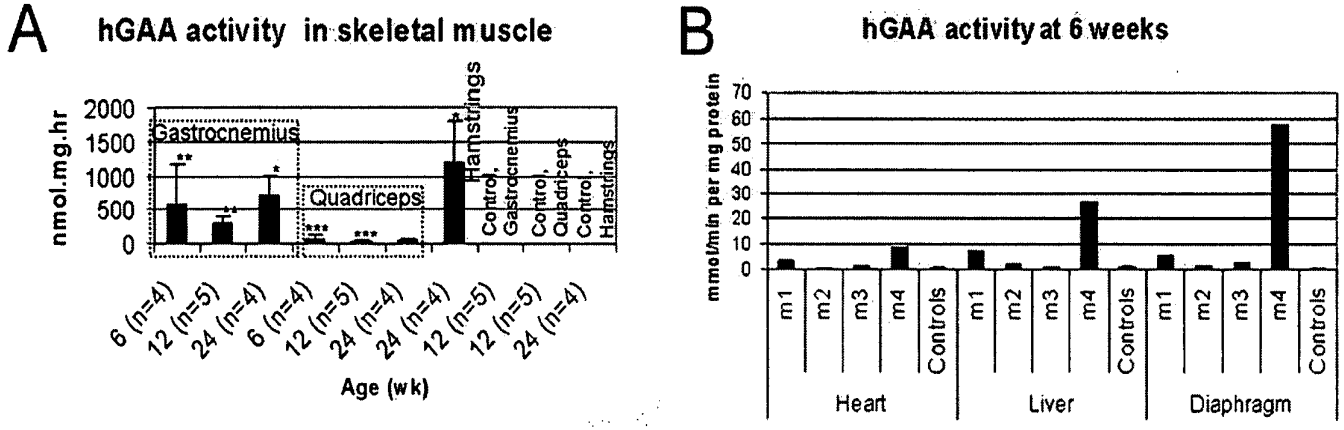
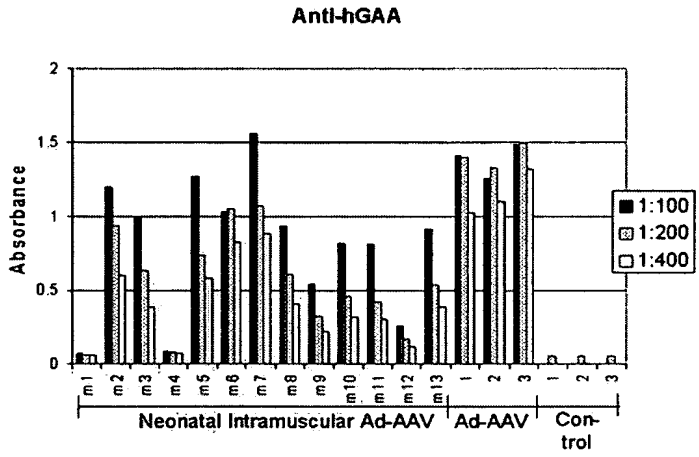
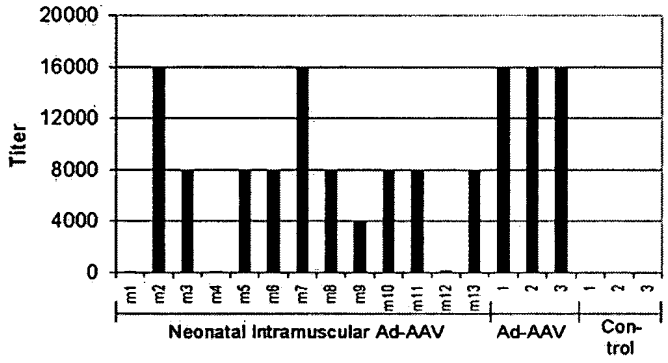

A
hGAA activity in skeletal muscle
nmol.mg.hr
2000
1500
1000
500
0
Gastrocnemius
Quadriceps
Hamstrings
Contro, Gastrocnemius
Contro, Quadriceps
Contro, Hamstrings
6 (n=4)
12 (n=5)
24 (n=4)
6 (n=4)
12 (n=5)
24 (n=4)
24 (n=4)
12 (n=5)
12 (n=5)
24 (n=4)
Age (wk)
B
hGAA activity at 6 weeks
mmol/min per mg protein
70
60
50
40
30
20
10
0
m1
m2
m3
m4
Controls
Heart
Liver
Diaphragm

FIG. 2

A


Anti-hGAA
Absorbance
2
1.5
1
0.5
0
1:100
1:200
1:400
m1 m2 m3 m4 m5 m6 m7 m8 m9 m10 m11 m12 m13 1 2 3 1 2 3
Neonatal Intramuscular Ad-AAV
Ad-AAV
Con-trol

B


Anti-hGAA
Titer
20000
16000
12000
8000
4000
0
m1 m2 m3 m4 m5 m6 m7 m8 m9 m10 m11 m12 m13 1 2 3 1 2 3
Neonatal Intramuscular Ad-AAV
Ad-AAV
Con-trol

C

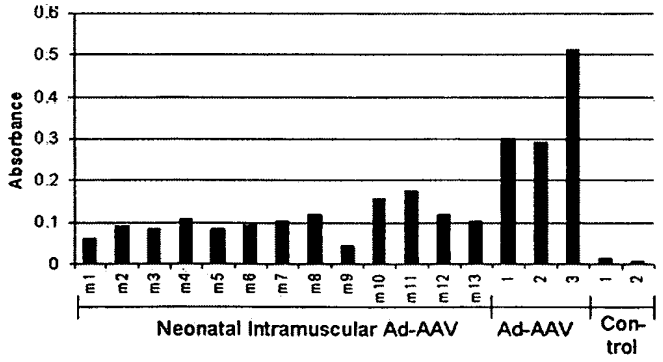
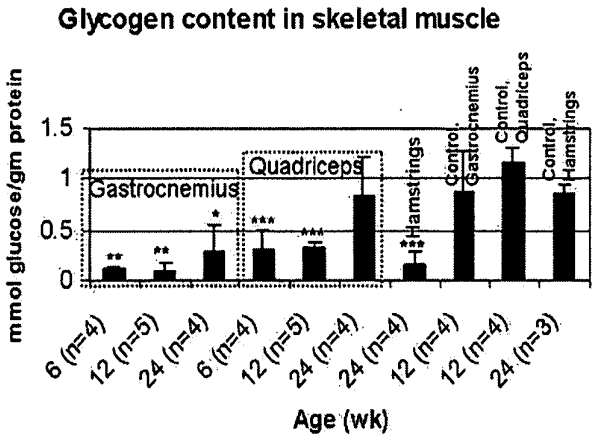

Anti-Ad
Absorbance
0.6
0.5
0.4
0.3
0.2
0.1
0
m1 m2 m3 m4 m5 m6 m7 m8 m9 m10 m11 m12 m13 1 2 3 1 2
Neonatal Intramuscular Ad-AAV
Ad-AAV
Con-trol

FIG. 3

A


Glycogen content in skeletal muscle
mmol glucose/gm protein
1.5
1
0.5
0
Gastrocnemius
Quadriceps
Hamstrings
Control, Gastrocnemius
Control, Quadriceps
Control, Hamstrings
6 (n=4)
12 (n=5)
24 (n=4)
6 (n=4)
12 (n=5)
24 (n=4)
24 (n=4)
12 (n=4)
12 (n=4)
24 (n=3)
Age (wk)

B

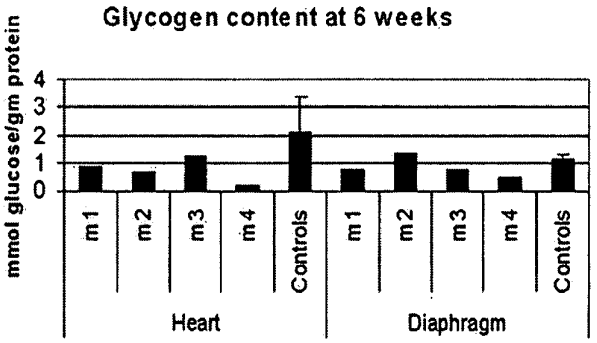

Glycogen content at 6 weeks
mmol glucose/gm protein
4
3
2
1
0
m1
m2
m3
m4
Controls
m1
m2
m3
m4
Controls
Heart
Diaphragm

FIG. 4

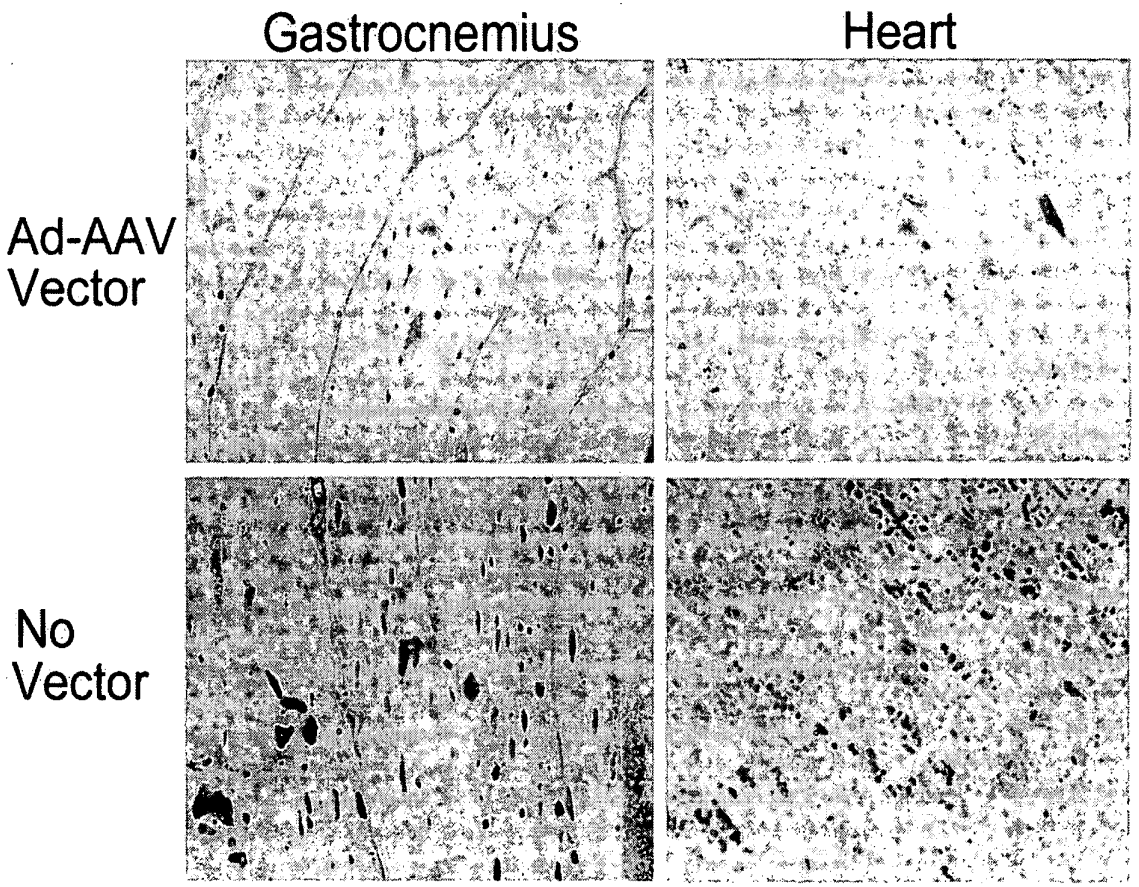
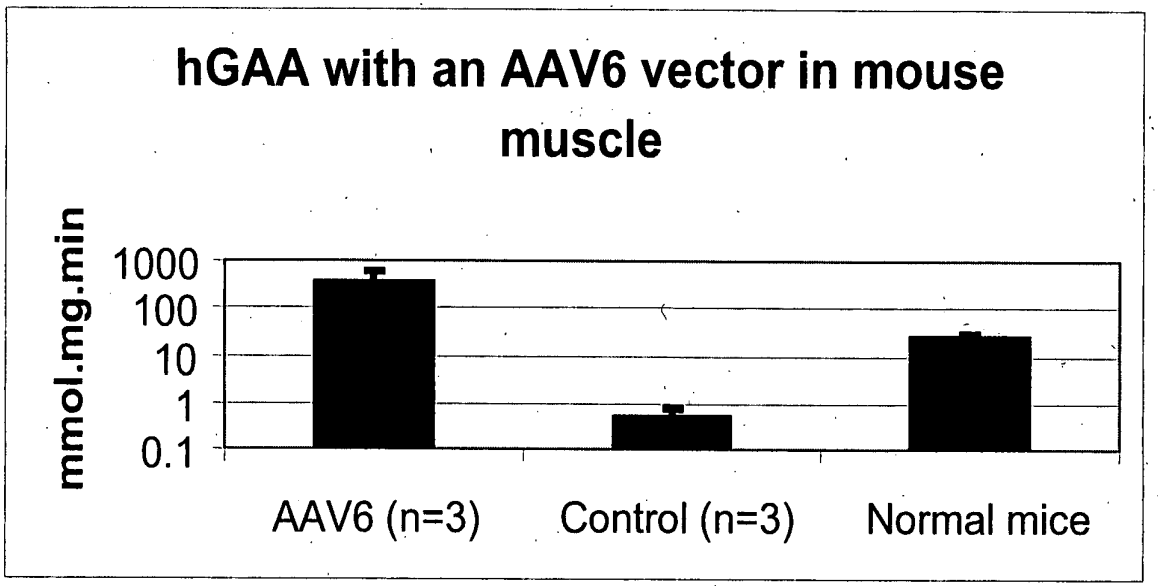


FIG. 5


hGAA with an AAV6 vector in mouse muscle
mmol.mg.min
1000
100
10
1
0.1
AAV6 (n=3)
Control (n=3)
Normal mice

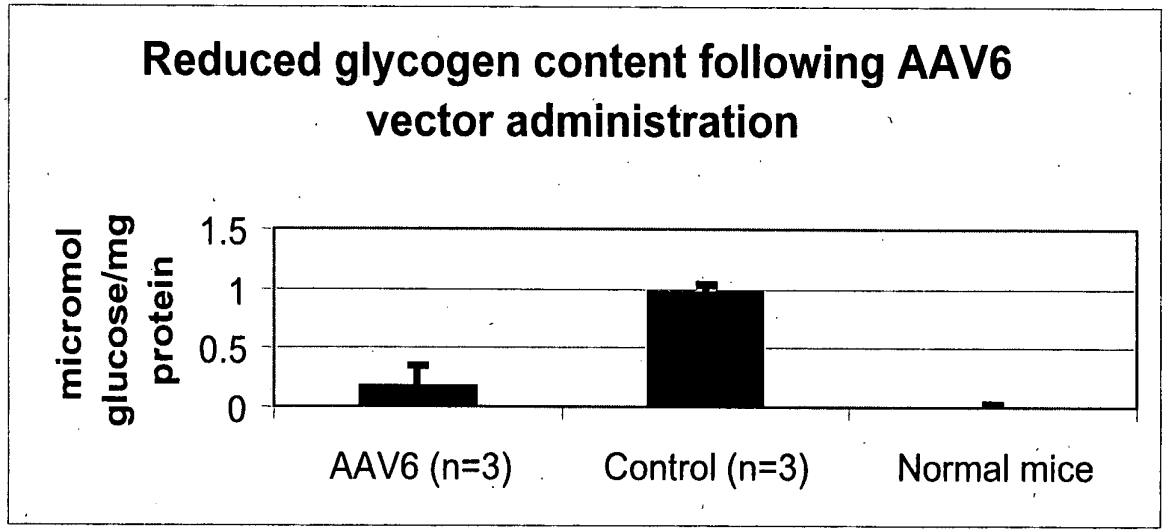

Reduced glycogen content following AAV6 vector administration
micromol glucose/mg protein
1.5
1
0.5
0
AAV6 (n=3)
Control (n=3)
Normal mice

FIG. 6

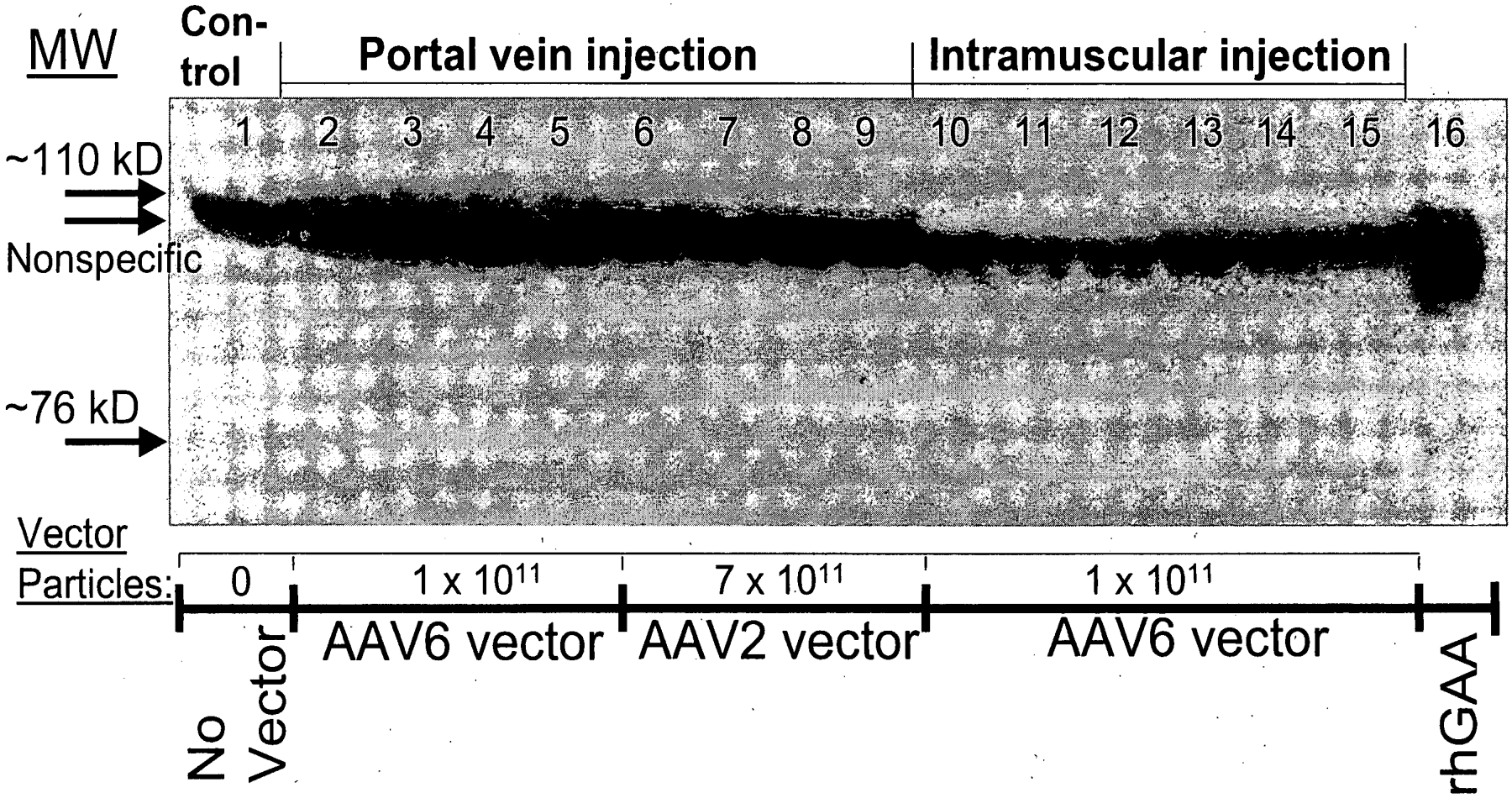

MW
Con-trol
Portal vein injection
Intramuscular injection
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
~110 kD
Nonspecific
~76 kD
Vector Particles:
0
1 x 10^11
7 x 10^11
1 x 10^11
No Vector
AAV6 vector
AAV2 vector
AAV6 vector
rhGAA

FIG. 7

Figure 8

The full-length human GAA cDNA sequence (3846 bp, Genebank number: NM_000152).

1 gcgcctgcgc gggaggccgc gtcacgtgac ccaccgcggc cccgccccgc gacgagctcc
61 cgccggtcac gtgacccgcc tctgcgcgcc cccgggcacg accccggagt ctccgcgggc
121 ggccagggcg cgcgtgcgcg gaggtgagcc gggccggggc tgcggggctt ccctgagcgc
181 gggccgggtc ggtggggcgg tcggctgccc gcgccggcct ctcagttggg aaagctgagg
241 ttgtcgccgg ggccgcgggt ggaggtcggg gatgaggcag caggtaggac agtgacctcg
301 gtgacgcgaa ggaccccggc cacctctagg ttctcctcgt ccgcccgttg ttcagcgagg
361 gaggctctgg gcctgccgca gctgacgggg aaactgaggc acggagcggg cctgtaggag
421 ctg tccaggc catctccaac c**atg**ggagtg aggcacccgc cctgctccca ccggctcctg
481 gccgtctgcg ccctcgtgtc cttggcaacc gctgcactcc tggggcacat cctactccat
541 gatttcctgc tggttccccg agagctgagt ggctcctccc cagtcctgga ggagactcac
601 ccagctcacc agcagggagc cagcagacca gggccccggg atgcccaggc acaccccggc
661 cgtcccagag cagtgcccac acagtgcgac gtccccccca acagccgctt cgattgcgcc
721 cctgacaagg ccatcaccca ggaacagtgc gaggcccgcg gctgctgcta catccctgca
781 aagcaggggc tgcagggagc ccagatgggg cagccctggt gcttcttccc acccagctac
841 cccagctaca agctggagaa cctgagctcc tctgaaatgg gctacacggc caccctgacc
901 cgtaccaccc ccaccttctt ccccaaggac atcctgaccc tgcggctgga cgtgatgatg
961 gagactgaga accgcctcca cttcacgatc aaagatccag ctaacaggcg ctacgaggtg
1021 cccttggaga ccccgcgtgt ccacagccgg gcaccgtccc cactctacag cgtggagttc
1081 tccgaggagc ccttcggggt gatcgtgcac cggcagctgg acggccgcgt gctgctgaac
1141 acgacggtgg cgcccctgtt ctttgcggac cagttccttc agctgtccac ctcgctgccc
1201 tcgcagtata tcacaggcct cgccgagcac ctcagtcccc tgatgctcag caccagctgg
1261 accaggatca ccctgtggaa ccgggacctt gcgcccacgc ccggtgcgaa cctctacggg
1321 tctcaccctt tctacctggc gctggaggac ggcgggtcgg cacacggggt gttcctgcta
1381 aacagcaatg ccatggatgt ggtcctgcag ccgagccctg cccttagctg gaggtcgaca
1441 ggtgggatcc tggatgtcta catcttcctg ggcccagagc ccaagagcgt ggtgcagcag
1501 tacctggacg ttgtgggata cccgttcatg ccgccatact ggggcctggg cttccacctg
1561 tgccgctggg gctactcctc caccgctatc acccgccagg tggtggagaa catgaccagg
1621 gcccacttcc ccctggacgt ccaatggaac gacctggact acatggactc ccggagggac
1681 ttcacgttca acaaggatgg cttccgggac ttcccggcca tggtgcagga gctgcaccag
1741 ggcggccggc gctacatgat gatcgtggat cctgccatca gcagctcggg ccctgccggg
1801 agctacaggc cctacgacga gggtctgcgg agggggttt tcatcaccaa cgagaccggc
1861 cagccgctga ttgggaaggt atggcccggg tccactgcct tccccgactt caccaacccc
1921 acagccctgg cctggtggga ggacatggtg gctgagttcc atgaccaggt gcccttcgac
1981 ggcatgtgga ttgacatgaa cgagccttcc aacttcatca gaggctctga ggacggctgc
2041 cccaacaatg agctggagaa cccaccctac gtgcctgggg tggttggggg gaccctccag
2101 gcggccacca tctgtgcctc cagccaccag tttctctcca cacactacaa cctgcacaac
2161 ctctacggcc tgaccgaagc catcgcctcc cacagggcgc tggtgaaggc tcgggggaca
2221 cgcccatttg tgatctcccg ctcgaccttt gctggccacg gccgatacgc cggccactgg
2281 acgggggacg tgtggagctc ctgggagcag ctcgcctcct ccgtgccaga aatcctgcag

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2341 tttaacctgc tgggggtgcc tctggtcggg gccgacgtct gcggcttcct gggcaacacc
2401 tcagaggagc tgtgtgtgcg ctggacccag ctgggggcct tctacccctt catgcggaac
2461 cacaacagcc tgctcagtct gccccaggag ccgtacagct tcagcgagcc ggcccagcag
2521 gccatgagga aggccctcac cctgcgctac gcactcctcc cccacctcta cacactgttc
2581 caccaggccc acgtcgcggg ggagaccgtg gcccggcccc tcttcctgga gttccccaag
2641 gactctagca cctggactgt ggaccaccag ctcctgtggg gggaggccct gctcatcacc
2701 ccagtgctcc aggccgggaa ggccgaagtg actggctact tccccttggg cacatggtac
2761 gacctgcaga cggtgccaat agaggccctt ggcagcctcc cacccccacc tgcagctccc
2821 cgtgagccag ccatccacag cgaggggcag tgggtgacgc tgccggcccc cctggacacc
2881 atcaacgtcc acctccgggc tgggtacatc atccccctgc agggccctgg cctcacaacc
2941 acagagtccc gccagcagcc catggccctg gctgtggccc tgaccaaggg tggagaggcc
3001 cgaggggagc tgttctggga cgatggagag agcctggaag tgctggagcg aggggcctac
3061 acacaggtca tcttcctggc caggaataac acgatcgtga atgagctggt acgtgtgacc
3121 agtgagggag ctggcctgca gctgcagaag gtgactgtcc tgggcgtggc cacggcgccc
3181 cagcaggtcc tctccaacgg tgtccctgtc tccaacttca cctacagccc cgacaccaag
3241 gtcctggaca tctgtgtctc gctgttgatg ggagagcagt ttctcgtcag ctggtgt**tag**
3301 ccgggcggag tgtgttagtc tctccagagg gaggctggtt ccccagggaa gcagagcctg
3361 tgtgcgggca gcagctgtgt gcgggcctgg gggttg ***catg tgtcacctgg agctgggcac***
3421 taaccattcc aagccgccgc atcgcttgtt tccacctcct gggccggggc tctggccccc
3481 aacgtgtcta ggagagcttt ctccctagat cgcactgtgg gccggggcct ggagggctgc
3541 tctgtgttaa taagattgta aggtttgccc tcctcacctg ttgccggcat gcgggtagta
3601 ttagccaccc ccctccatct gttcccagca ccggagaagg gggtgctcag gtggaggtgt
3661 ggggtatgca cctgagctcc tgcttcgcgc ctgctgctct gccccaacgc gaccgcttcc
3721 cggctgccca gagggctgga tgcctgccgg tccccgagca agcctgggaa ctcaggaaaa
3781 ttcacaggac ttgggagatt ctaaatc tta agtgcaatta tttt**aataaa** aggggcattt
3841 ggaatc

Figure 9

5' and 3' deleted GAA

1 ..... 409 410 g cctgtaggag
421 ctg tccaggc catctccaac c**atg**ggagtg aggcacccgc cctgctccca ccggctcctg
481 gccgtctgcg ccctcgtgtc cttggcaacc gctgcactcc tggggcacat cctactccat
541 gatttcctgc tggttccccg agagctgagt ggctcctccc cagtcctgga ggagactcac
601 ccagctcacc agcagggagc cagcagacca gggccccggg atgcccaggc acaccccggc
661 cgtcccagag cagtgcccac acagtgcgac gtccccccca acagccgctt cgattgcgcc
721 cctgacaagg ccatcaccca ggaacagtgc gaggcccgcg gctgctgcta catccctgca
781 aagcaggggc tgcagggagc ccagatgggg cagccctggt gcttcttccc acccagctac
841 cccagctaca agctggagaa cctgagctcc tctgaaatgg gctacacggc caccctgacc
901 cgtaccaccc ccaccttctt ccccaaggac atcctgaccc tgcggctgga cgtgatgatg
961 gagactgaga accgcctcca cttcacgatc aaagatccag ctaacaggcg ctacgaggtg
1021 cccttggaga ccccgcgtgt ccacagccgg gcaccgtccc cactctacag cgtggagttc
1081 tccgaggagc ccttcggggt gatcgtgcac cggcagctgg acggccgcgt gctgctgaac
1141 acgacggtgg cgcccctgtt ctttgcggac cagttccttc agctgtccac ctcgctgccc
1201 tcgcagtata tcacaggcct cgccgagcac ctcagtcccc tgatgctcag caccagctgg
1261 accaggatca ccctgtggaa ccgggacctt gcgcccacgc ccggtgcgaa cctctacggg
1321 tctcaccctt tctacctggc gctggaggac ggcgggtcgg cacacggggt gttcctgcta
1381 aacagcaatg ccatggatgt ggtcctgcag ccgagccctg cccttagctg gaggtcgaca
1441 ggtgggatcc tggatgtcta catcttcctg ggcccagagc ccaagagcgt ggtgcagcag
1501 tacctggacg ttgtgggata cccgttcatg ccgccatact ggggcctggg cttccacctg
1561 tgccgctggg gctactcctc caccgctatc acccgccagg tggtggagaa catgaccagg
1621 gcccacttcc ccctggacgt ccaatggaac gacctggact acatggactc ccggagggac
1681 ttcacgttca acaaggatgg cttccgggac ttcccggcca tggtgcagga gctgcaccag
1741 ggcggccggc gctacatgat gatcgtggat cctgccatca gcagctcggg ccctgccggg
1801 agctacaggc cctacgacga gggtctgcgg agggggggttt tcatcaccaa cgagaccggc
1861 cagccgctga ttgggaaggt atggcccggg tccactgcct tccccgactt caccaacccc
1921 acagccctgg cctggtggga ggacatggtg gctgagttcc atgaccaggt gcccttcgac
1981 ggcatgtgga ttgacatgaa cgagccttcc aacttcatca gaggctctga ggacggctgc
2041 cccaacaatg agctggagaa cccaccctac gtgcctgggg tggttggggg gaccctccag
2101 gcggccacca tctgtgcctc cagccaccag tttctctcca cacactacaa cctgcacaac
2161 ctctacggcc tgaccgaagc catcgcctcc cacagggcgc tggtgaaggc tcgggggaca
2221 cgcccatttg tgatctcccg ctcgaccttt gctggccacg gccgatacgc cggccactgg
2281 acgggggacg tgtggagctc ctgggagcag ctcgcctcct ccgtgccaga aatcctgcag
2341 tttaacctgc tgggggtgcc tctggtcggg gccgacgtct gcggcttcct gggcaacacc
2401 tcagaggagc tgtgtgtgcg ctggacccag ctgggggcct tctacccctt catgcggaac
2461 cacaacagcc tgctcagtct gccccaggag ccgtacagct tcagcgagcc ggcccagcag

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2521 gccatgagga aggccctcac cctgcgctac gcactcctcc cccacctcta cacactgttc
2581 caccaggccc acgtcgcggg ggagaccgtg gcccggcccc tcttcctgga gttccccaag
2641 gactctagca cctggactgt ggaccaccag ctcctgtggg gggaggccct gctcatcacc
2701 ccagtgctcc aggccgggaa ggccgaagtg actggctact tccccttggg cacatggtac
2761 gacctgcaga cggtgccaat agaggccctt ggcagcctcc cacccccacc tgcagctccc
2821 cgtgagccag ccatccacag cgaggggcag tgggtgacgc tgccggcccc cctggacacc
2881 atcaacgtcc acctccgggc tgggtacatc atccccctgc agggccctgg cctcacaacc
2941 acagagtccc gccagcagcc catggccctg gctgtggccc tgaccaaggg tggagaggcc
3001 cgaggggagc tgttctggga cgatggagag agcctggaag tgctggagcg aggggcctac
3061 acacaggtca tcttcctggc caggaataac acgatcgtga atgagctggt acgtgtgacc
3121 agtgagggag ctggcctgca gctgcagaag gtgactgtcc tgggcgtggc cacggcgccc
3181 cagcaggtcc tctccaacgg tgtccctgtc tccaacttca cctacagccc cgacaccaag
3241 gtcctggaca tctgtgtctc gctgttgatg ggagagcagt ttctcgtcag ctggtgt**tag**
3301 ccgggcggag tgtgttagtc tctccagagg gaggctggtt ccccagggaa gcagagcctg
3361 tgtgcgggca gcagctgtgt gcgggcctgg gggttg 3397.....3807
3808 tta agtgcaatta ttt**taataaa** aggggcatttggaatc

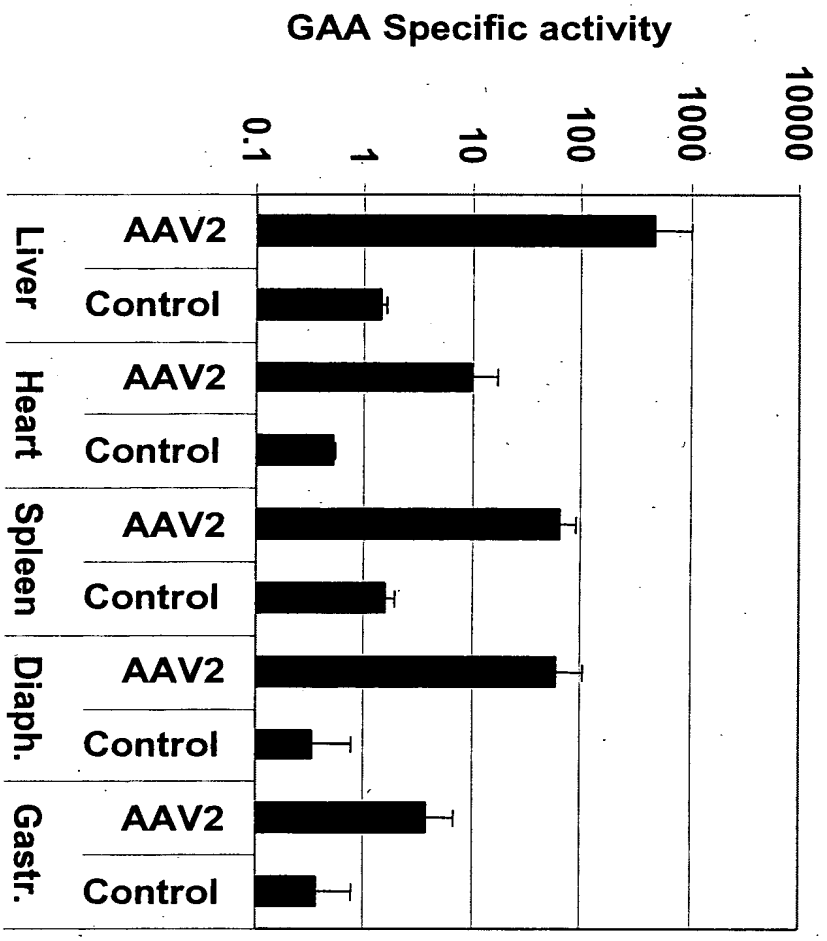
GAA Specific activity
0.1
1
10
100
1000
10000
Liver
AAV2
Control
Heart
AAV2
Control
Spleen
AAV2
Control
Diaph.
AAV2
Control
Gastr.
AAV2
Control

FIG. 10

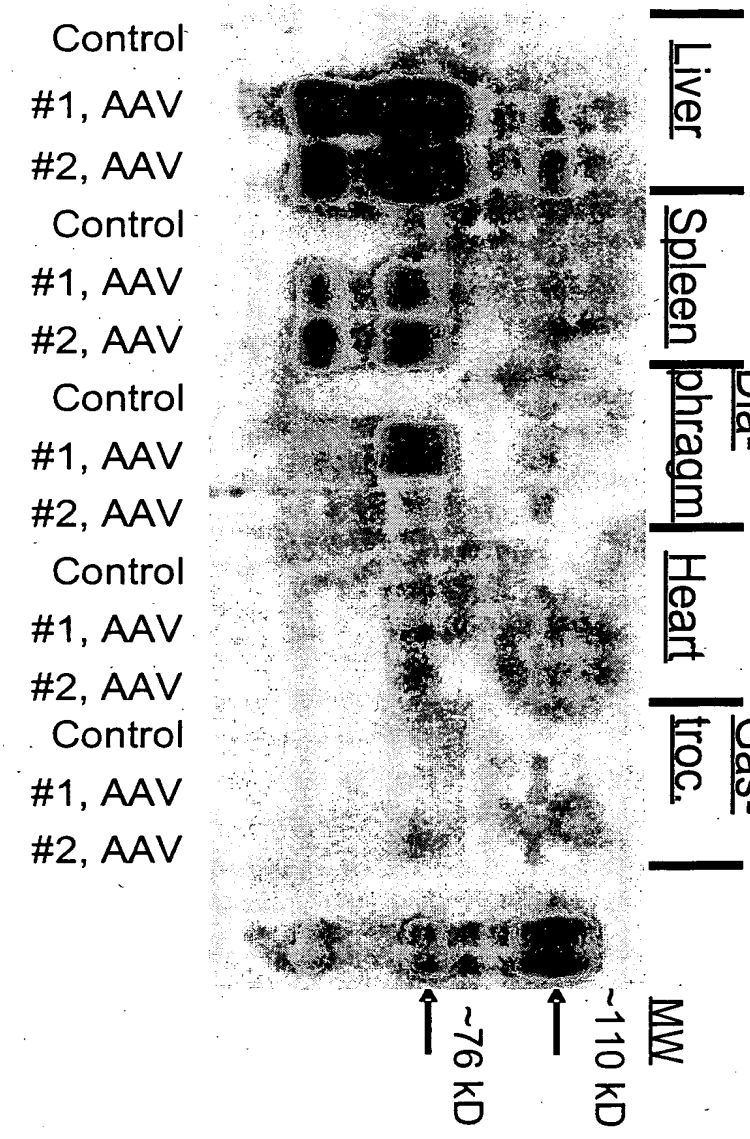
Control
#1, AAV
#2, AAV
Control
#1, AAV
#2, AAV
Control
#1, AAV
#2, AAV
Control
#1, AAV
#2, AAV
Control
#1, AAV
#2, AAV
Liver
Spleen
Dia-phragm
Heart
Gas-troc.
MW
~110 kD
~76 kD

FIG. 11

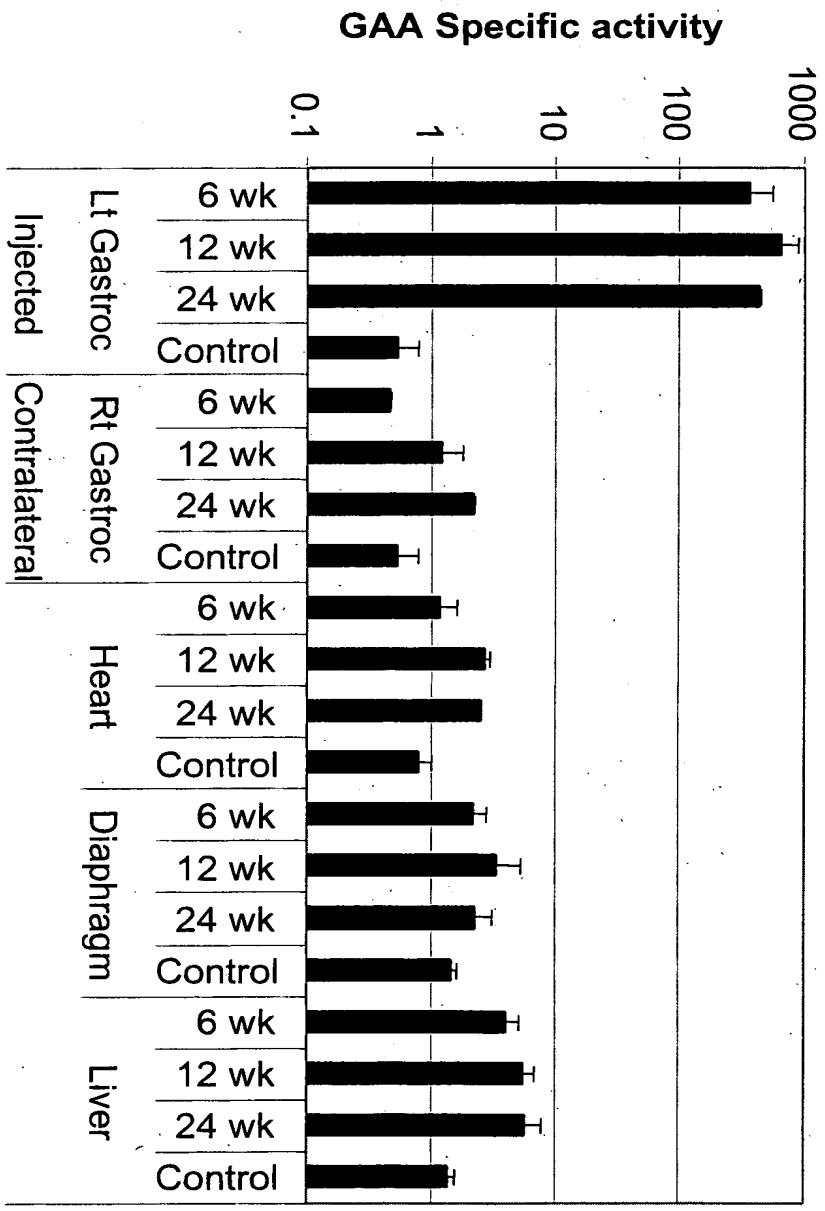
GAA Specific activity
0.1
1
10
100
1000
Injected
Contralateral
Lt Gastroc
Rt Gastroc
Heart
Diaphragm
Liver
6 wk
12 wk
24 wk
Control

FIG. 12

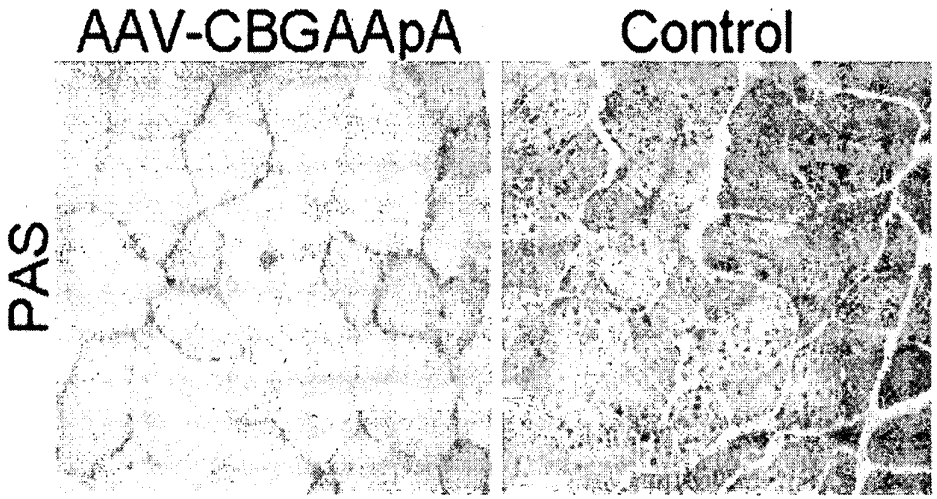

FIG. 13

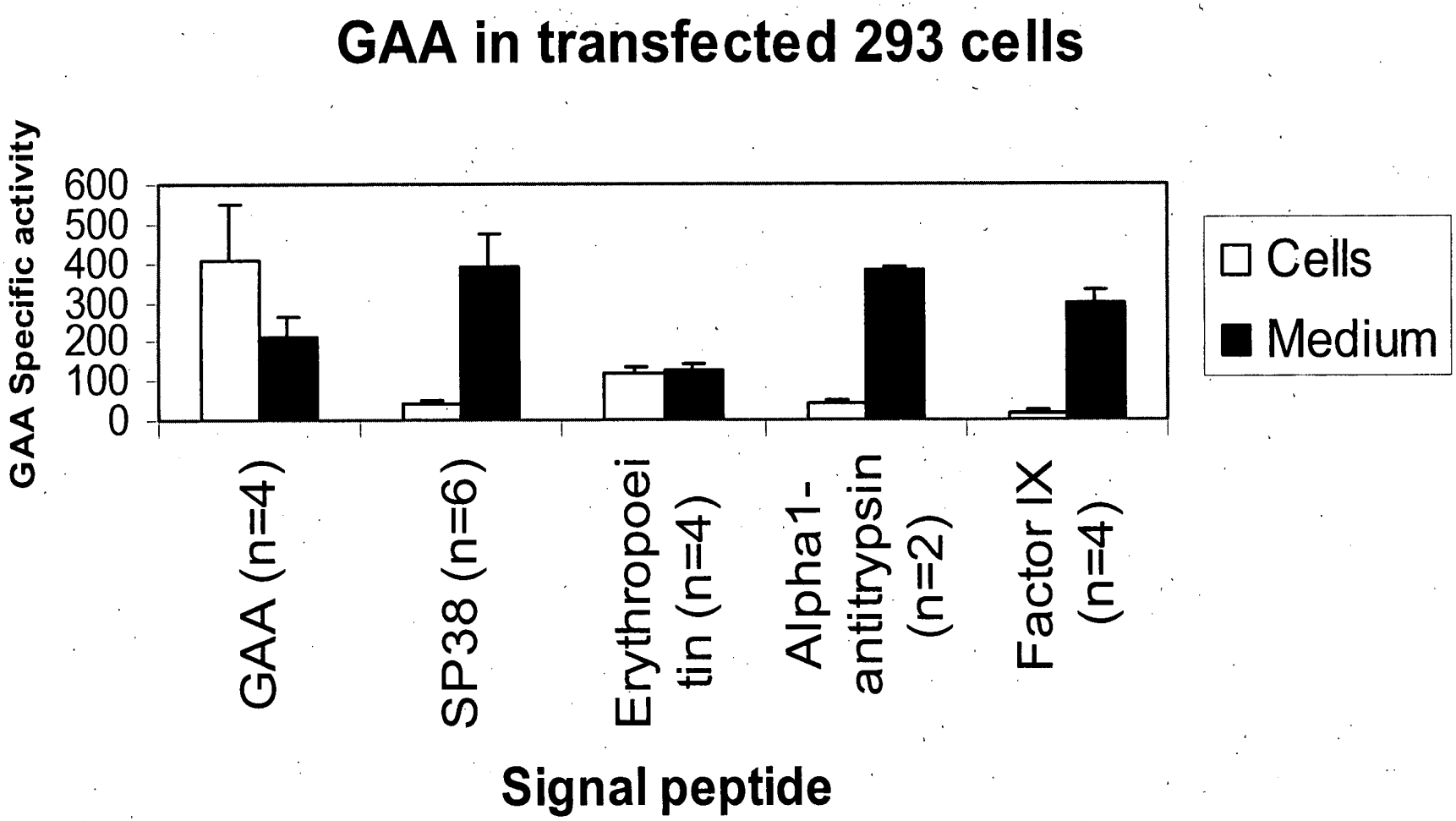
GAA in transfected 293 cells
GAA Specific activity
600
500
400
300
200
100
0
Cells
Medium
GAA (n=4)
SP38 (n=6)
Erythropoei tin (n=4)
Alpha1-antitrypsin (n=2)
Factor IX (n=4)
Signal peptide

FIG. 14

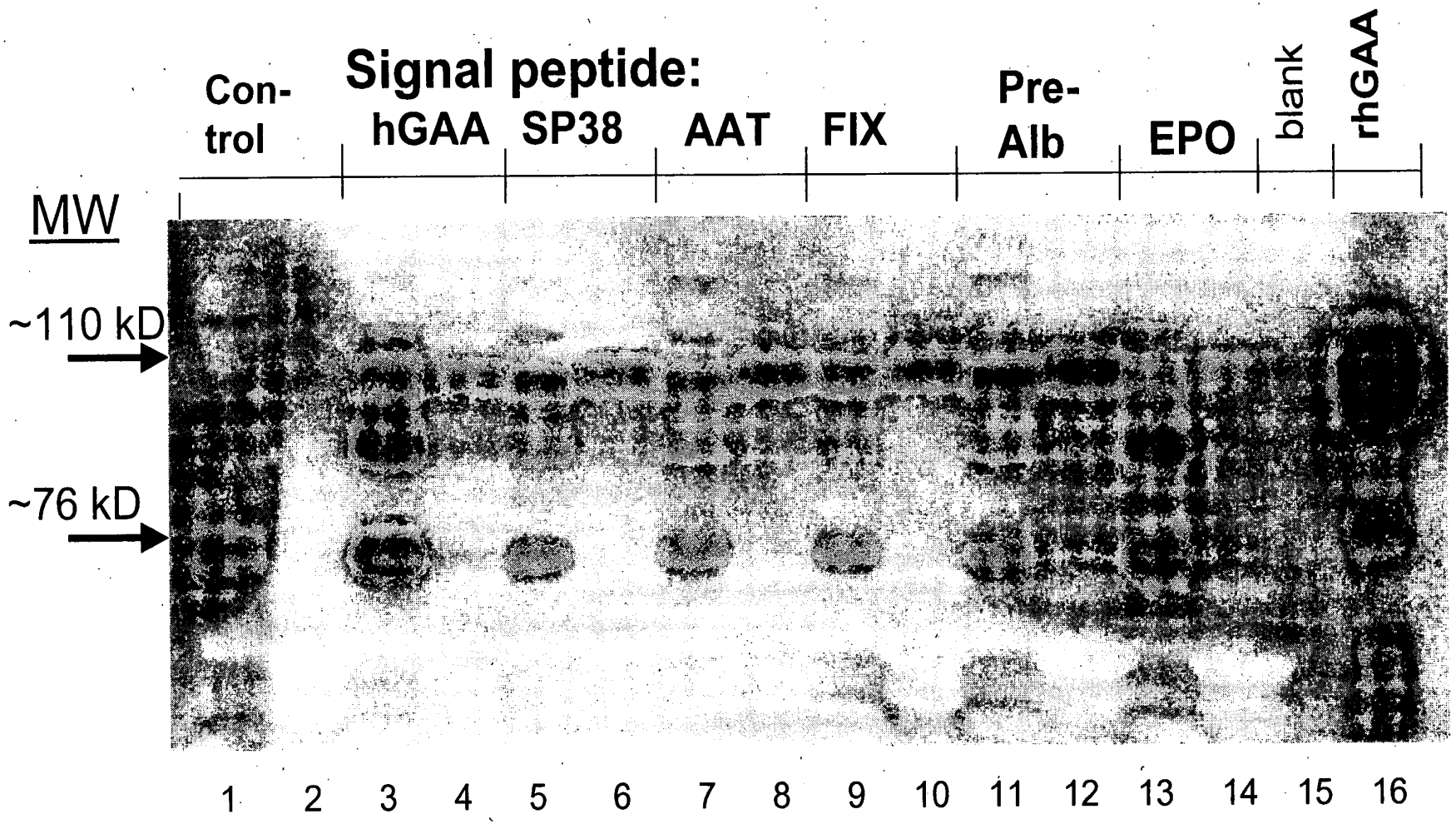
Signal peptide:
Con-trol
hGAA
SP38
AAT
FIX
Pre-Alb
EPO
blank
rhGAA
MW
~110 kD
~76 kD
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

FIG. 15

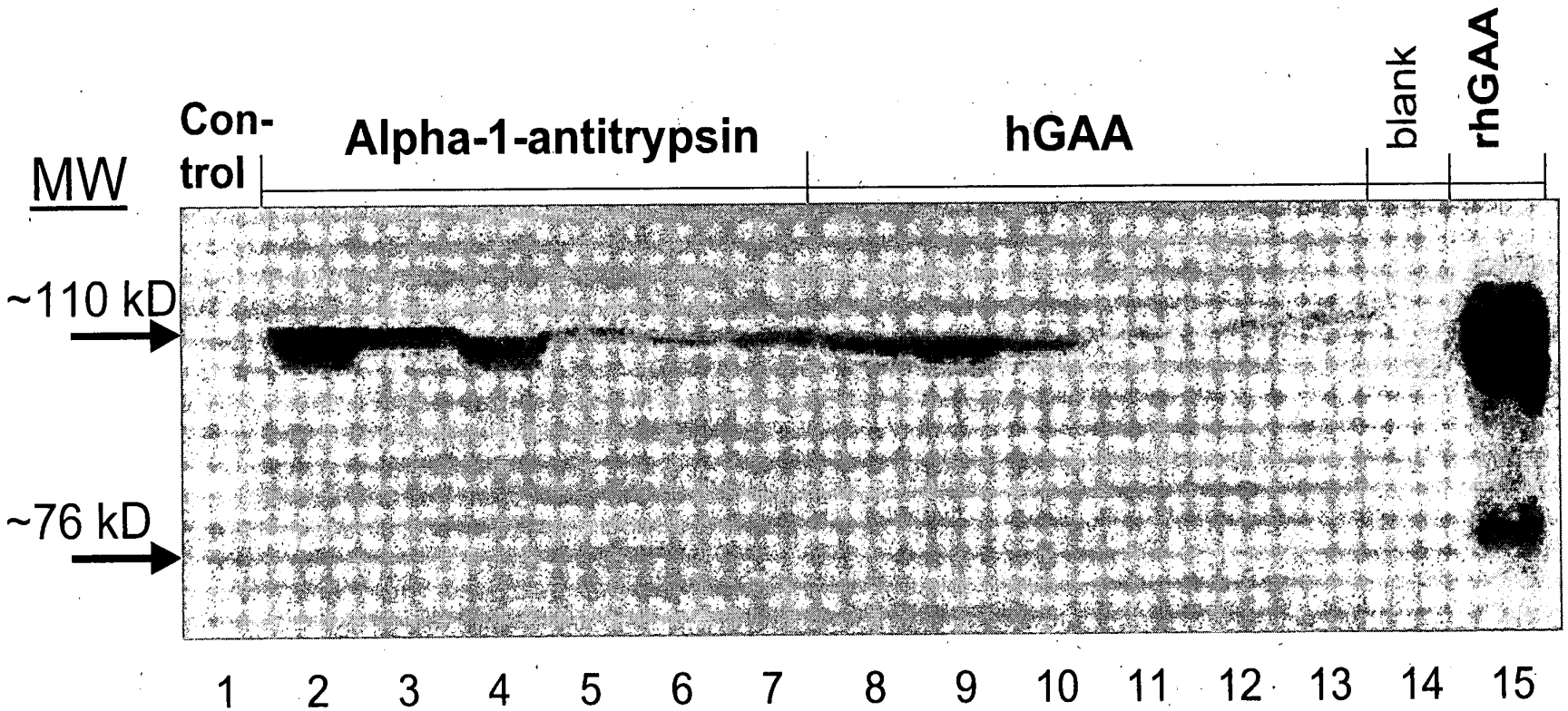
MW
Con-
trol
Alpha-1-antitrypsin
hGAA
blank
rhGAA
~110 kD
~76 kD
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

FIG. 16

FIG. 17

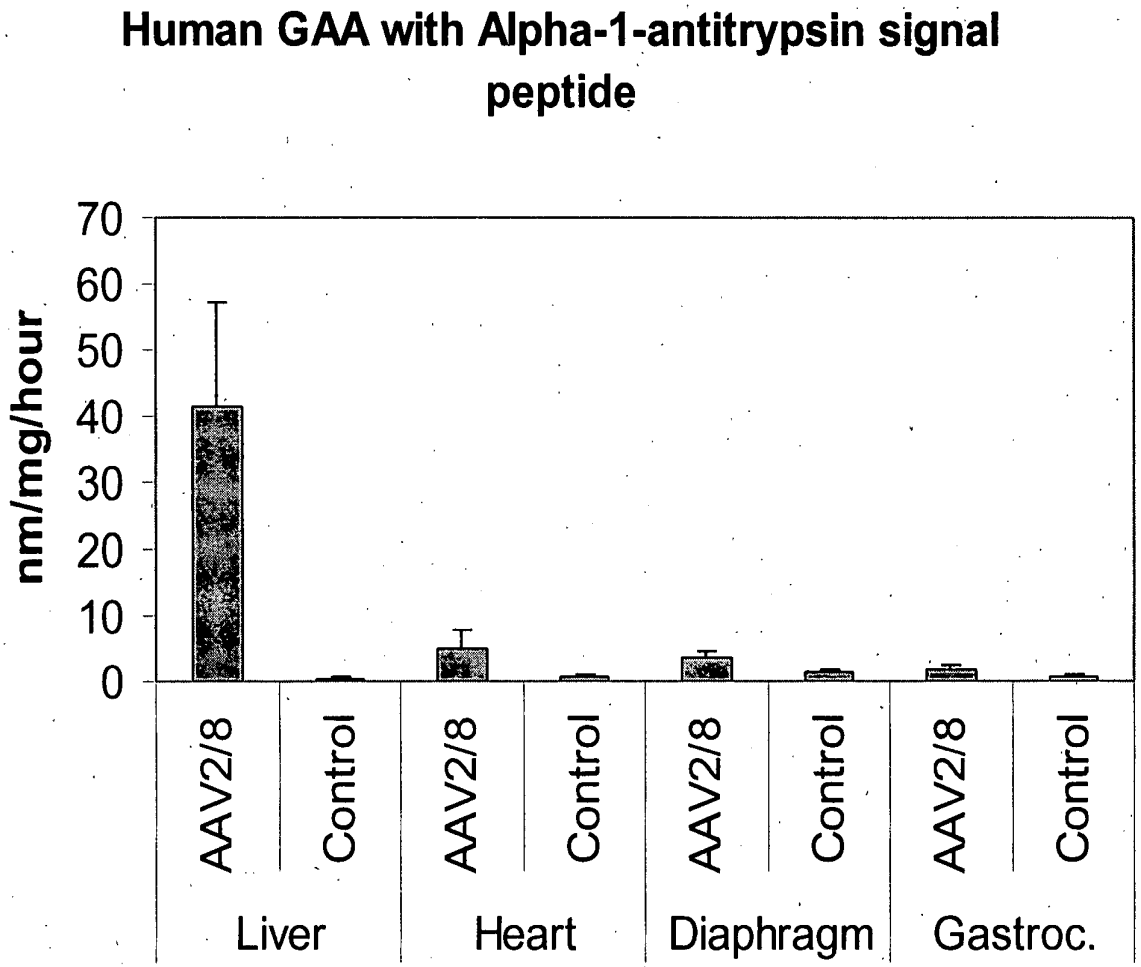
Human GAA with Alpha-1-antitrypsin signal peptide
nm/mg/hour
70
60
50
40
30
20
10
0
AAV2/8
Control
AAV2/8
Control
AAV2/8
Control
AAV2/8
Control
Liver
Heart
Diaphragm
Gastroc.

FIG. 18